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Development and Validation of RP-HPLC Method for the Simultaneous Estimation of Rabeprazole and Levosulpiride in Combined Tablet Dosage Form

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ABSTRACT

A rapid and precise reverse phase high performance liquid chromatographic method has been developed for the validated of Levosulpiride and Rabeprazole, in its pure form as well as in tablet dosage form. Chromatography was carried out on a Targetsil C18 (4.5×250mm) 5µ column using a mixture of Methanol: Phosphate Buffer pH 3.9 (55:45v/v) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 255nm. The retention time of the Rabeprazole and Levosulpiride was 2.051, 2.452±0.02min respectively. The method produce linear responses in the concentration range of 1-5µg/ml of Rabeprazole and 100-500µg/ml of Levosulpiride. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords Rabeprazole, Levosulpiride, RP-HPLC, validation.

INTRODUCTION

Rabeprazole Sodium is an antiulcer drug in the class of proton pump inhibitors. It inhibits the H+, K+ ATPase of the coating gastric cells. Chemically Rabeprazole is 2 ({[4-(3-methoxypropoxy)-3- methylpyridin-2-yl] methane} sulfinyl)-1H-1, 3 benzodiazole. Levosulpiride is a substituted benzamide derivative and a selective dopamine D2 antagonist with antipsychotic and antidepressant activity. Chemically Levosulpiride is 5- (aminosulfonyl) -N- (1-ethyl- 2-pyrrolidinyl) methyl+- 2-methoxy benzamide.Literature survey reveals that certain chromatographic methods were reported for simultaneous estimation of Rabeprazole and Levosulpiride. In view of the need for a suitable RP-HPLC method for routine analysis of Rabeprazole and Levosulpiride in formulations, attempts were made to develop simple, precise and accurate analytical method for simultaneous estimation of Rabeprazole and Levosulpiride and extend it for their determination in formulation.

EXPERIMENTAL WORK

Hplc Method Development:

Preparation of standard solution:Accurately weigh and transfer 10 mg of Rabeprazole and Levosulpirideworking standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol.Further pipette 0.03ml of Rabeprazole and 3.0ml of Levosulpiride from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure: Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization: Initially the mobile phase tried was Methanol: Water with varying proportions. Finally, the mobile phase was optimized to Methanol: Phosphate Buffer pH 3.9 in proportion 55:45 v/v respectively.

Optimization of Column:The method was performed with various column TargetsilC18 (4.5×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

Chromatographic Conditions:

Instrument used : Shimadzu LC-10 AT VP.
Temperature : Ambient
Mobile phase: Methanol: Phosphate Buffer pH 3.9 (55:45v/v)
Flow rate : 1ml/min
Wavelength : 284nm
Injection volume : 10 µl
Run time : 8 min

Validation

Preparation of Phosphate buffer pH 3.9:Accurately weighed 5.8 grams of KH_2PO_4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.9.

Preparation of mobile phase: Accurately measured 550 ml (55%) of Methanol and 450ml of Buffer (45%) were mixed and degassed in digital ultrasonicator for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation:The Mobile phase was used as the diluent.

VALIDATION PARAMETERS

Specificity Study Of Drug:

Preparation of Standard Solution:Accurately weigh and transfer 10 mg of Rabeprazole and 10mg of Levosulpirideworking standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 0.03ml of Rabeprazole and 3.0ml of Levosulpiride from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Preparation of Sample Solution:Take average weight of Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight ofRabeprazole andLevosulpiride sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Further pipette 0.03ml of Rabeprazole and 3.0ml of Levosulpiride from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure: Inject the three replicate injections of standard and sample solutions and calculate the assay by using formula:

$$\%\ \text{ASSAY} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

PREPARATION OF DRUGSOLUTIONS FOR LINEARITY:Accurately weigh and transfer 10 mg of Rabeprazole and 10mg of Levosulpirideworking standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Preparation of Level – I (1µg/ml of Rabeprazole & 100µg/ml of Levosulpiride): Pipette out 0.01ml of Rabeprazole and 1.0ml of Levosulpiride stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – II (2µg/ml of Rabeprazole & 200µg/ml of Levosulpiride): Pipette out0.02ml of Rabeprazole and 2.0 ml of Levosulpiride stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – III (3µg/ml of Rabeprazole & 300µg/ml of Levosulpiride): Pipette out 0.03ml of Rabeprazole and 3.0ml of Levosulpiride stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – IV (4µg/ml of Rabeprazole & 400µg/ml of Levosulpiride): Pipette out 0.04ml of Rabeprazole and 4.0ml of Levosulpiride stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Preparation of Level – V (5µg/ml of Rabeprazole & 500µg/ml of Levosulpiride): Pipette out 0.05ml of Rabeprazole and 5.0ml of Levosulpiride stock solutions was take in a 10ml of volumetric flask dilute up to the mark with diluent.

Procedure: Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient.

PRECISION

Repeatability
Preparation of Rabeprazole and Levosulpiride Product Solution for Precision: Accurately weigh and transfer 10 mg of Rabeprazole and 10mg of Levosulprideworking standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 0.03ml of Rabeprazole and 3.0ml of Levosulpiride from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Intermediate Precision: To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different days by maintaining same conditions.

Procedure:
DAY 1:The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.
DAY 2:The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found to be within the specified limits.

Accuracy:
For preparation of 50% Standard stock solution: Accurately weigh and transfer 10 mg of Rabeprazole and 10mg of Levosulprideworking standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 0.015ml of Rabeprazole and 1.5ml of Levosulpiride from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 100% Standard stock solution: Accurately weigh and transfer 10 mg of Rabeprazole and 10mg of Levosulprideworking standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 0.03ml of Rabeprazole and 3.0ml of Levosulpiride from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

For preparation of 150% Standard stock solution: Accurately weigh and transfer 10 mg of Rabeprazole and 10mg of Levosulprideworking standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).Further pipette 0.045ml of Rabeprazole and 4.5ml of Levosulpiride from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

Procedure:Inject the Three replicate injections of individual concentrations (50%, 100%, 150%) were made under the optimized conditions. Recorded the chromatograms and measured the peak responses. Calculate the Amount found and Amount added for Rabeprazole and Levosulprideand calculate the individual recovery and mean recovery values.

Robustness:
The analysis was performed in different conditions to find the variability of test results. The following conditions are checked for variation of results..

For preparation of Standard solution: Accurately weigh and transfer 10 mg of Rabeprazole and 10mg of Levosulprideworking standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)Further pipette 0.03ml of Rabeprazole and 3.0ml of Levosulpiride from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluents.

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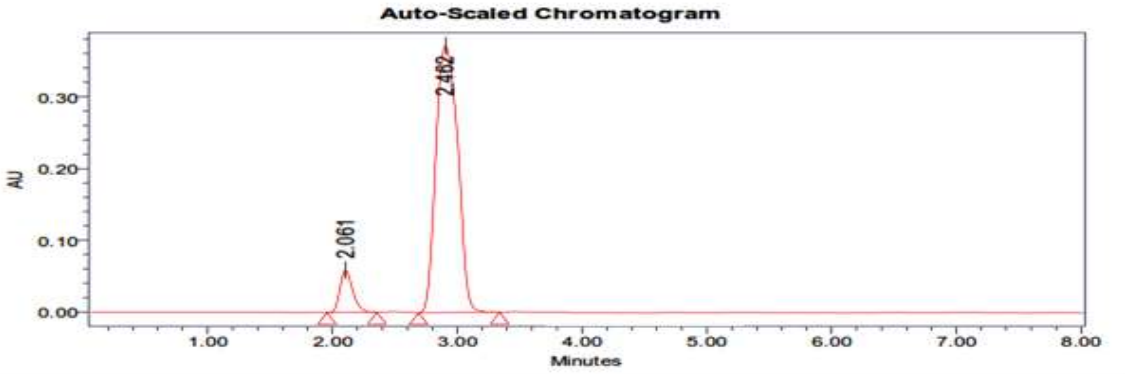
Effect of Variation of flow conditions:The sample was analyzed at 0.9 ml/min and 1.1 ml/min instead of 1ml/min, remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded Effect of Variation of mobile phase organic composition:The sample was analyzed by variation of mobile phase i.e. Methanol: Buffer was taken in the ratio and 50:50, 50:40 instead (55:45), remaining conditions are same. 10µl of the above sample was injected and chromatograms were recorded.

RESULTS



Optimized Chromatogram

S. No	Peak name	R_t	Area	Height	USP Tailing	USP plate count
1	Rabeprazole	2.051	247392	58952	1.2	7243
2	Levosulpiride	2.452	3530855	371748	1.1	3389

Peak Results For Optimized

S.No	Name	Rt	Area	Height	USP Tailing	USP plate count
1	Rabeprazole	2.058	244102	89282	1.2	5949
2	Levosulpiride	2.489	3357555	575552	1.0	5855
3	Rabeprazole	2.070	240052	88021	1.2	5851
4	Levosulpiride	2.491	3371553	575999	1.0	5808
5	Rabeprazole	2.057	243230	88882	1.2	5879
6	Levosulpiride	2.489	3354001	570315	1.0	5823

Table: Peak results for Assay sample

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Rabeprazole	2.055	249584	12079	5343	1.0
2	Rabeprazole	2.054	249595	12058	5473	1.2
3	Rabeprazole	2.054	245325	11949	5473	1.1
4	Rabeprazole	2.055	249815	11811	5389	1.1
5	Rabeprazole	2.057	249892	11735	5180	1.0
Mean			249082.5			
Std. Dev			1543.954			
% RSD			0.51985			

Table: Results of repeatability for Rabeprazole:

S.No	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Levosulpiride	2.485	3233700	59095	5554	1.2
2	Levosulpiride	2.484	3241323	57552	5524	1.3
3	Levosulpiride	2.482	3245927	57213	5440	1.3
4	Levosulpiride	2.483	3245927	57095	5411	1.4
5	Levosulpiride	2.483	3222194	54353	5250	1.4
Mean			3237814			
Std. Dev			10050.52			
% RSD			0.310722			

Table: Results of repeatability for Levosulpiride :

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Rabeprazole	2.055	242721	11323	5272	1.21
2	Rabeprazole	2.055	240155	11554	5158	1.15
3	Rabeprazole	2.055	240945	11887	5310	1.14
4	Rabeprazole	2.055	240385	11938	5275	1.19
5	Rabeprazole	2.059	249920	11552	5078	1.10
6	Rabeprazole	2.057	240820	11750	5225	1.17
Mean			243991			
Std. Dev			4541.97			
% RSD			1.5			

Table: Results of Intermediate precisionDay 1 for Rabeprazole

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Levosulpiride	2.477	3325309	54143	5149	1.25
2	Levosulpiride	2.478	3323780	53740	5127	1.21
3	Levosulpiride	2.483	3328190	54791	5507	1.28
4	Levosulpiride	2.485	3329035	55098	5759	1.28
5	Levosulpiride	2.489	3325958	52379	5709	1.30
5	Levosulpiride	2.483	3327725	54779	5755	1.35
Mean			3325558			
Std. Dev			1985.541			
% RSD			0.059589			

Table: Results of Intermediate precision Day 1 for Levosulpiride

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Rabeprazole	2.057	249499	11594	5240	1.2
2	Rabeprazole	2.059	240991	11357	5130	1.2
3	Rabeprazole	2.058	240431	11878	5135	1.2
4	Rabeprazole	2.059	241330	11748	5257	1.2
5	Rabeprazole	2.057	240519	11830	5222	1.2
5	Rabeprazole	2.057	240470	11475	5982	1.2
Mean			242205.7			
Std. Dev			3590.034			
% RSD			1.48222			

Table: Results of Intermediate precision Day 2 for Rabeprazole

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Levosulpiride	2.485	3425979	53353	5700	1.3
2	Levosulpiride	2.484	3445541	54454	5553	1.3
3	Levosulpiride	2.495	3430505	53532	5855	1.3
4	Levosulpiride	2.484	3430952	55157	5854	1.3
5	Levosulpiride	2.490	3431575	55223	5942	1.3
5	Levosulpiride	2.490	3429187	58578	5544	1.3
Mean			3433812			
Std. Dev			7041.409			
% RSD			0.205051			

Table: Results of Intermediate precisionDay 2 for Levosulpiride

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	124575.7	15	15.1	101%	100.4%
100%	242005.3	30	30.1	100.5%	
150%	357449	45	44.9	99.7%	

Table : Accuracy results for Rabeprazole

%Concentration (at specification Level)	Area	Amount Added (µg/ml)	Amount Found (µg/ml)	% Recovery	Mean Recovery
50%	1595259	18.75	18.71	99.8%	99.2%
100%	3351551	37.5	37.2	99.4%	
150%	4975094	55.25	55.47	98.5%	

Table: The Accuracy results for Levosulpiride

LIMIT OF DETECTION
Rabeprazole:
=3.3 × 1750.8/78322=0.07µg/ml
Levosulpiride :
=3.3 × 51155/11150=18.0µg/ml
LIMIT OF QUANTITATION
Rabeprazole:
=10×1750.8/78322= 0.2µg/ml
Levosulpiride :
=10 × 51155/11150= 54.8µg/ml

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	247392	2.051	7243	1.2
Less Flow rate of 0.9 mL/min	59214	2.257	4713	1.3
More Flow rate of 1.1 mL/min	388838	1.854	4740	1.2
Less organic phase	445528	2.155	4709	1.2
More organic phase	59404	1.957	5590	1.4

Table: Results For Robustnessrabeprazole

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	3530855	2.452	3389	1.1
Less Flow rate of 0.9 mL/min	527373	2.590	5275	1.0
More Flow rate of 1.1 mL/min	4353129	2.284	5511	1.0
Less organic phase	3955572	2.590	5550	1.0
More organic phase	527708	2.390	5273	1.0

Table: Results For Robustnesslevosulpiride

Summary

The analytical method was developed by studying different parameters.First of all, maximum absorbance was found to be at 255nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was Targetsil C_{18} because it was giving good peak. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time.

Mobile phase is Methanol: Phosphate Buffer pH 3.9 (55:45v/v) was fixed due to good symmetrical peak. So this mobile phase was used for the proposed study. Run time was selected to be 8min because analyze gave peak around 2.051, 2.452 ±0.02min respectively and also to reduce the total run time.The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range 1-5µg/ml of Rabeprazole and 100-500µg/ml of Levosulpiride of the target concentration.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Rabeprazole and Levosulpiride in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps.

The %RSD values were within 2 and the method was found to be precise.The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Rabeprazole and Levosulpiride in bulk drug and in Pharmaceutical dosage forms.

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